

EXHIBIT D

Serial assessment of cell-free circulating tumor DNA (ctDNA) to assess treatment effect and minimal residual disease during neoadjuvant and adjuvant therapy in colorectal cancer



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Abstract #O-016

Introduction

- In stage I – III colon cancer, ctDNA identifies patients with minimal or “molecular” residual disease (MRD) following therapy completion^{1,2}
- Patients with post-resection MRD may benefit from additional adjuvant therapy and/or close surveillance
- Longitudinal ctDNA assessment may provide insight into dynamic changes in ctDNA and better inform therapy decisions
- We previously demonstrated a plasma only integrated genomic and epigenomic ctDNA assay (Figure 1) has a high positive predictive value for colon cancer recurrence in patients who have completed standard of care therapy (Figure 2; Table 1)^{3,4}

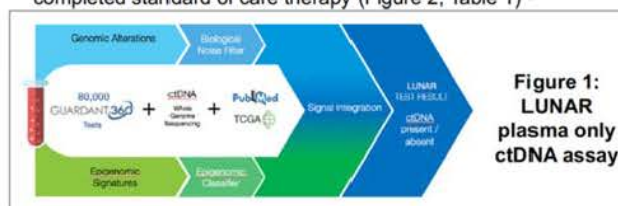


Figure 1:
LUNAR
plasma only
ctDNA assay

Methods

- Serial plasma samples collected from 43 people with resectable colon cancer a median of 31 days post-completion of each intervention with a median 27ng (range 10-100) of cfDNA input (Table 2; Figure 3)
- Single blood sample assay (Figure 1)
 - Analysis of both genomic and epigenomic signals
 - 100% detection at 0.1% variant allele fraction (VAF) for 30ng DNA input
 - Variants as low as 0.01% VAF reported
 - In-silico filtering of non-tumor derived alterations
 - No need for tumor
- Definitions:
 - Persistent ctDNA: “ctDNA detected” following completion of therapy
 - Cleared ctDNA: “ctDNA detected” followed by “ctDNA not detected” after completion of therapy
 - Negative ctDNA: “ctDNA not detected” at all timepoints

Figure 3: Patient Consort

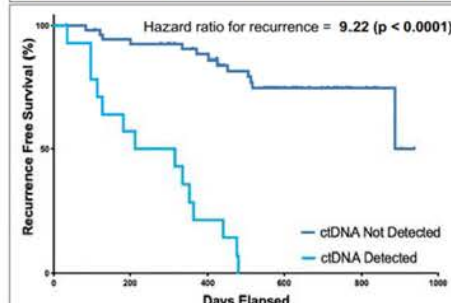
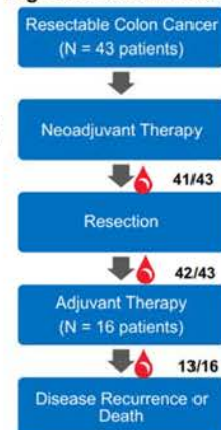


Figure 2 and Table 1: ctDNA Detection Post-Completion of Standard of Care Therapy

Assay Performance by Analysis		
	Genomic (N)	Integrated Genomic and Epigenomic (N)
PPV (N of patients with ctDNA detected who recurred)	100% (11 / 11)	100% (14 / 14)
NPV (N of patients with ctDNA not detected who were recurrence free)	72% (42 / 58)	76% (42 / 55)
Sensitivity for recurrence within one year of surgery	56% (9 / 16)	69% (11 / 16)
Specificity for recurrence within one year of surgery	96% (51 / 53)	94% (50 / 53)

Table 2: Cohort Demographics		Number of patients	(%)
Gender	Female	15	35%
	Male	28	65%
Median Age at diagnosis (range)		56 years (31 – 81)	
Stage (at resection)	0 - II	13	30%
	III	11	26%
	IV	19	44%

Results

- In this cohort of treated patients, the addition of epigenomic assessment improves ctDNA detection (Figure 4)
- Patients with persistent ctDNA were significantly more likely to experience disease recurrence ($p < 0.0001$) in a shorter time period (Table 2; Figure 5)

Figure 4: ctDNA results

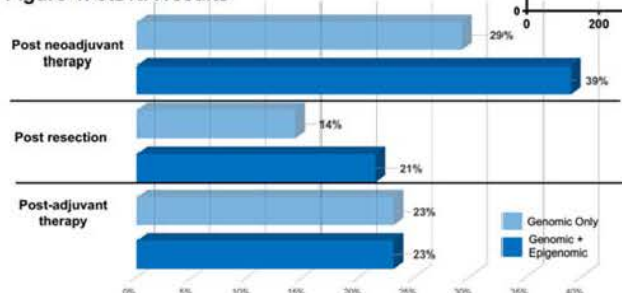
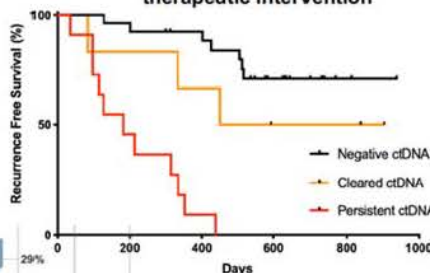


Figure 5: ctDNA persistence following therapeutic intervention



Conclusions

- Patients with persistent ctDNA following completion of therapy for colon cancer were significantly more likely to experience disease recurrence earlier
- ctDNA can be serially assessed following each therapeutic intervention
- Understanding dynamic changes in ctDNA status following each intervention can identify patients who may benefit from additional therapies
- Prospective interventional studies are ongoing to explore utilizing ctDNA to inform therapeutic decision making

Table 2: Dynamic changes in ctDNA status	Recurred	Recurrence Free	Median Time to Recurrence (days)
Persistent ctDNA	11	0	182
Cleared ctDNA	3	3	333
Negative ctDNA	7	19	NR* (median follow-up: 580 days)

¹Tie, et al. STM. 2016; ²Reinert, et al. JAMA Oncol. 2019; ³Hartwig, et al (2019). JCO. 37 (suppl abstr 3057); ⁴Parikh, et al (2019). JCO. 37 (suppl abstr 3602)